IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	**10/628,792**
Applicants	:	**Jon A. Wolff et al.**
Filed	:	**07/28/2003**
Art Unit	:	**1654**
Examiner	:	**Ha, Julie**
Docket No.	:	**Mirus.040.01**

For: **Delivery of Molecules and Complexes to Mammalian Cells In Vivo**

Commissioner of Patents
PO Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132

Dear Sir:

I, Julia Hegge, hereby declare as follows:

1. I have a Bachelor's degree in Biology and Medical Technology from Edgewood College and over 20 year experience in the Medical Technology field.
2. I am familiar with the above captioned application and with U.S. Patent 5,346,696.
3. I am familiar with injection of solution into the vasculature or various mammals.
4. I am the author of the attached statement regarding the injection parameters as taught by U.S. Patent 5,346,696.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

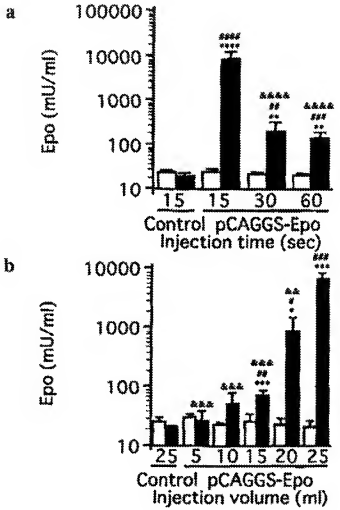
Julia Hegge 11/6/07
Julia Hegge date

Kim et al. (U.S. Patent 5,346,696) demonstrated receptor mediated targeting of asialo-glycoprotein conjugates to the liver. The liver targeting shown by Kim et al. is expected to be useful for molecules which are readily permeably through the discontinuous liver vasculature. However, the injection procedure taught by Kim et al., 1 ml injection into the tail vein of rat or 1 ml injection into an ear vein in rabbit, would be too small to cause an increase in vascular permeability in the liver. Below are experimental data correlating injection volume and injection rate with delivery of nucleic acids to the liver or muscle in rats. The data illustrate that injection of 1 ml into the tail vein or iliac artery of a rat is not sufficient to deliver nucleic acid to liver parenchymal or skeletal muscle cells.

In example 1, white bars indicate the background level of expression measure prior to delivery of the gene. Values equal to or less than these levels, within standard deviation, indicate no delivery. As seen in panel (b), injection of 5 ml or less into the tail vein of rats resulted in no delivery of the gene to liver cells.

Example 2 shows experimental data correlating injection rate and volume into the iliac artery with molecule delivery skeletal muscle. Injection of 2 ml or less failed to provide any delivery.

Example 1. Effect of injection parameters on serum Epo levels. Effects of (a) varying the injection time (15-60 s) with a constant volume (25 ml, n=6 in each group), and (b) varying the volume (5-25 ml) within a constant time (15 s, n=4 in each group), on the efficiency of gene transfer to rat liver . Epo levels were determined one week before (white bars) and one week after (black bars) injecting naked plasmid DNA encoding the EPO gene. 25 mls corresponded to about 100 ml/ kg animal weight (1 ml per 10 g animal weight).



Example 2. Effects of injection solution volume (a) and injection time (b) on the plasmid DNA delivery to in rat hindlimb muscles. Plasmid DNA encoding the Luciferase gene in various volumes (9.5 ml for (b)) of normal saline solution was injected into the iliac artery of adult Sprague-Dawley rats. Delivery, as measured by Luciferase activity in hindlimb muscle, was measured and is presented as the means of the total Luciferase activity. Numbers adjacent to time points indicate the number of animals (one limb per animal) assayed for each condition. Error bars indicate the standard error.